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A PILOT TEST TO CONTROL DOUGLAS-FIR TUSSOCK MOTHS BY AN AERIAL APPLICATION OF NUCLEAR POLYHEDROSIS VIRUS DURING 1965

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Region 1

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A PILOT TEST TO CONTROL DOUGLAS-FIR TUSSOCK MOTHS BY AN AERIAL APPLICATION OF NUCLEAR POLYHEDROSIS VIRUS DURING 1965

By
Scott Tunnock, Entomologist
Forest Pest Control Branch

SUMMARY

The effectiveness of a nuclear polyhedrosis virus obtained from diseased Douglas-fir tussock moth larvae was tested during the summer of 1965. A helicopter was used to spray 1,220 acres at the rate of 1 billion polyhedra in 1 gallon of water per acre when most larvae were second instars. Larvae, collected before treatment and at weekly intervals after treatment, were reared on artificial media in plastic cups at an insectary until they died from polyhedrosis, were killed by other agents, or emerged as adults.

Mortality from polyhedrosis was consistently higher in an untreated area than in a treated area. Therefore, the application of 1 billion polyhedra per acre did not significantly decrease the Douglas-fir tussock moth population.

Mortality from other diseases, parasites, and unknown agents was not above 17.6 percent during any of the collection periods. Hymenopterous and dipterous parasites caused most of this mortality. Five species of wasps killed 96.7 percent of the parasitized larvae.

Some adult emergence occurred in each collection. However, many adults were malformed, which indicated that they may have been infected with virus. No current egg masses could be found in treated and untreated areas during the fall of 1965.

INTRODUCTION

Outbreaks of the Douglas-fir tussock moth, Hemerocampa pseudotsugata McD., apparently occur at 4- to 6-year intervals in western North America. Stands of Douglas-fir and true fir trees are often severely defoliated, which causes considerable top-killing.

Populations of this moth began to increase in northern Idaho during 1961. By 1964 various numbers of tussock moth egg masses were found on 300,000 acres in Benewah and Latah Counties, Idaho. Heavy defoliation was predicted for 1965.

Evenden (1) indicated that a polyhedral virus caused considerable mortality during an outbreak in 1947 within 20,000 acres near Orofino, Idaho. Since then, nuclear polyhedrosis virus has been tested as a control agent and sprayed from the air to kill tussock moth larvae (4, 11).

In 1965, 1,220 acres northeast of Moscow, Idaho, were sprayed with virus as a pilot test. The main objectives of this pilot test were to:

1. Determine the effectiveness of applying 1 billion polyhedra per acre in 1 gallon of water.

2. Develop methods for mass rearing tussock moth larvae, inoculating them with virus, and processing polyhedral bodies from their cadavers.

3. Develop a sampling method for determining the amount of mortality caused by introducing virus from the air.

Assisting in this project were Kenneth W. Keefe, forester; and Frederick W. Honing, entomologist, who was detailed from Region 4, Ogden, Utah. Personnel from the Palouse Ranger District also cooperated during many facets of the test. Technical assistance was received from Dr. C. G. Thompson, Pacific Northwest Forest and Range Experiment Station.

MATERIALS AND METHODS

Rearing tussock moth larvae.--Egg masses collected from various infested areas in Idaho during the winter of 1965 were placed in Petri dishes (fig. 1) containing a piece of wet sponge. At room temperature, eggs hatched in 11 to 16 days. Emerging larvae were transferred to plastic shoe boxes (fig. 2) and fed larch foliage. The foliage was produced by inserting the ends of dormant larch twigs in buckets of water. Larch buds sprouted within 2 weeks when kept at room temperature. First instars preferred the more succulent larch foliage to previous year's fir needles. Also, larvae developed much faster through the first three instars when fed larch compared to those fed fir. Larval growth was fastest when the temperature was between 75° and 85° F. and the humidity was near 100 percent. Foliage was sprayed daily, because water droplets are necessary in the insect's diet. About 250 larvae were reared in each plastic box until they reached the third instar.

Inoculating larvae with virus.--Five hundred third instars were transferred to cardboard boxes (16 by 12 by $10\frac{1}{2}$ inches) with plastic on two sides and on the lid (fig. 3). The diet was changed from larch to Douglas-fir foliage, which was easier to obtain during the winter months, and was readily eaten by the late instars.

Larvae were infected by feeding them Douglas-fir foliage dipped in water containing 1 billion polyhedra per gallon. This dosage rate killed most of them during the last instar. Last instar cadavers yielded a great deal more polyhedra than third instars.

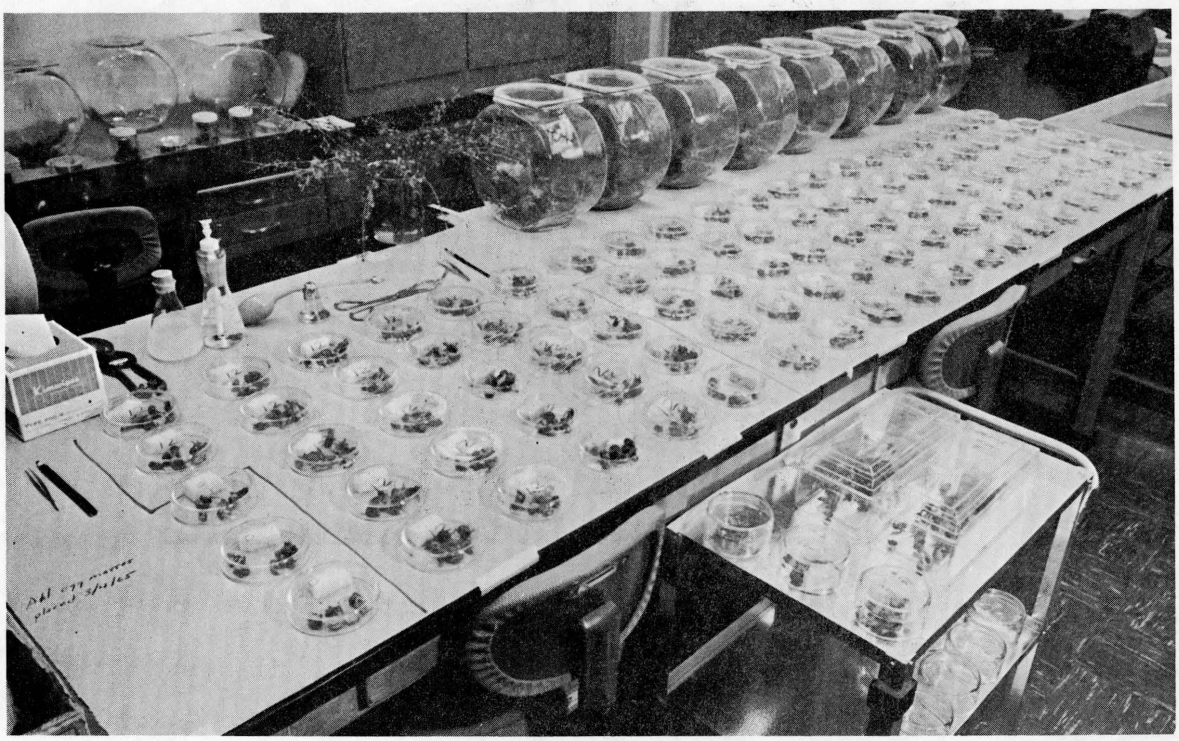


Figure 1.--Douglas-fir tussock moth egg masses being incubated in Petri dishes. Some emerging larvae were placed in goldfish bowls and fed larch foliage.

Figure 2.--Plastic shoe boxes in which first three instars were reared on larch foliage.

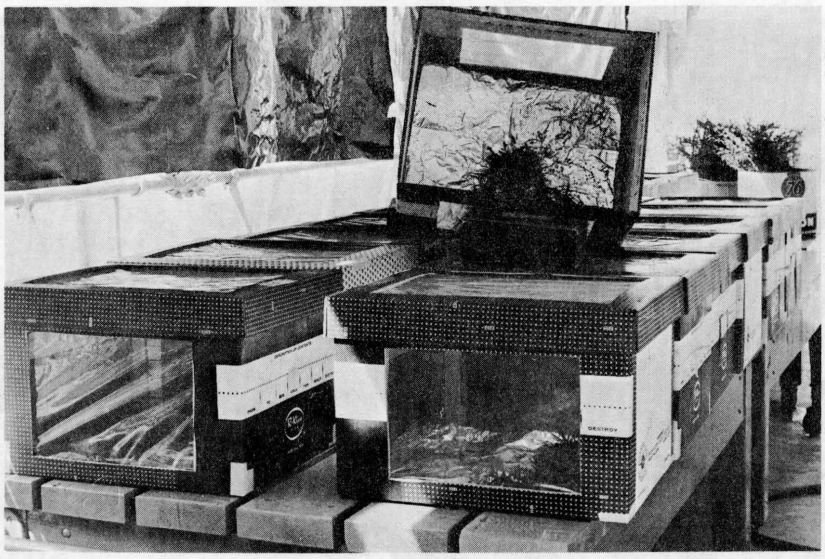


Figure 3.--Cardboard boxes with plastic windows in which last three instars were fed Douglas-fir foliage contaminated with polyhedrosis virus.

Figure 4.--Collecting dead larvae, killed by polyhedrosis, from the cardboard rearing cages.

Processing infected larvae.--Dead larvae were removed from the cardboard boxes (fig. 4), placed in dry, glass bottles, and stored at 4° C. Batches of cadavers were put in distilled water and mixed in a Waring blender. The blended material was filtered through 40-gage, plastic screen, through cheesecloth, and final-filtered through fine nylon cloth (fig. 5). Deposits on the filters were washed off with distilled water, blended again, and filtered as above to obtain residual polyhedra. The filtrate was stored in darkness at 4° C. (6). The number of polyhedra per milliliter of filtrate was determined with a hemacytometer slide. The filtrate was maintained at a pH of 7.0, since solutions having a pH of 4.0 to 5.0 and 8.0 to 8.5 are critical and can dissolve polyhedra (7).

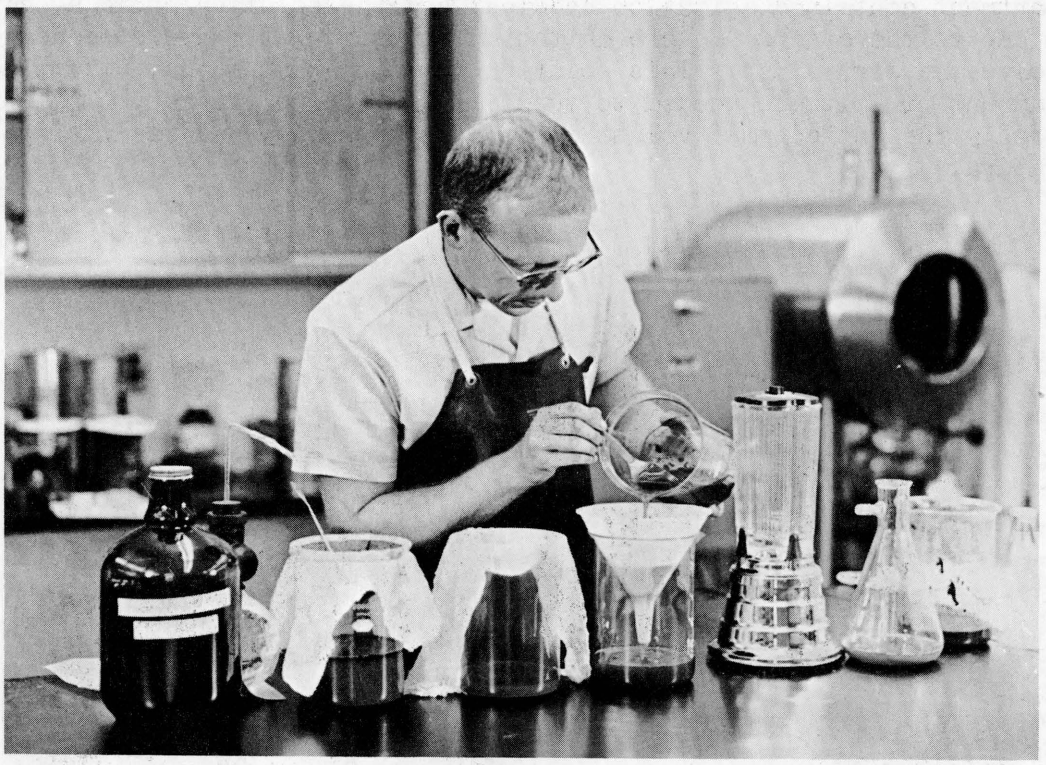


Figure 5.--Blending and filtering cadavers of diseased larvae to obtain a solution of polyhedra in distilled water.

Mixing spray solution.--As previously mentioned, batches of filtrate contained various numbers of polyhedra per milliliter, but to formulate 1 gallon of spray solution containing 1 billion polyhedra, ingredients in the following amounts were used:

0.90 gallon of nonchlorinated water.

.10 gallon of light corn sirup (evaporation inhibitor).

.01 gallon of Leucophor C 6208 (U) (fluorescent tracer).

2.00 milliliters of filtrate (if filtrate contained 500 million polyhedra per milliliter).

Proportionate amounts of the first three ingredients to make 1,500 gallons of solution were poured into a mixing tank. The mixing unit (fig. 6) consisted of a 1,800-gallon tank divided into two 900-gallon compartments. Each compartment contained agitation paddles, and the solution could be circulated from one to the other. Severe agitation and circulation were needed to dissolve the corn sirup. This solution was mixed thoroughly before adding the amount of filtrate needed to supply 1 billion polyhedra per gallon. Each day, only the anticipated volume of spray solution needed was charged with polyhedra.

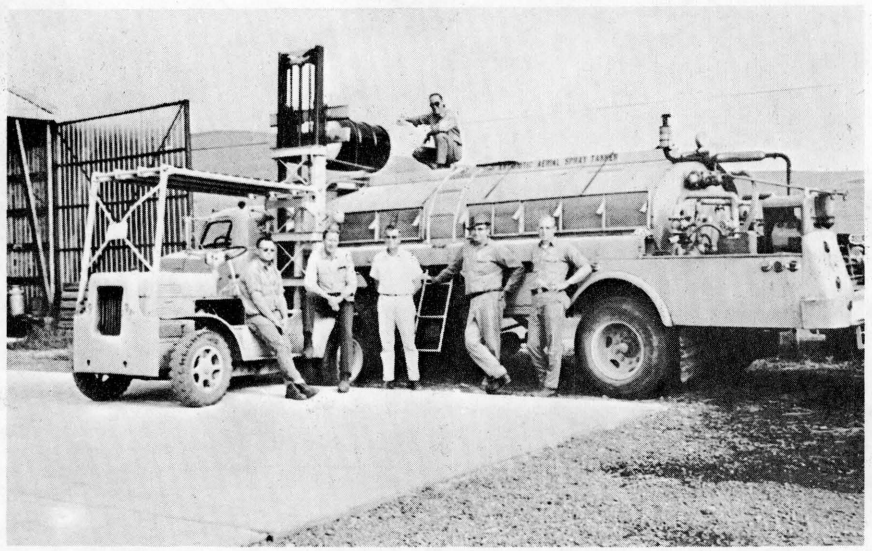


Figure 6.--The 1,800-gallon unit used to mix the virus spray solution. Corn sirup was obtained from the 50-gallon drum on hoist.

Treatment.--A Hiller helicopter (fig. 7) applied the spray solution. Its maximum load was 70 gallons, and its spray system was calibrated to deliver 1 gallon per acre at 60 miles per hour.

Figure 7.--Filling the helicopter's spray tanks with spray solution.

Observations made during the spraying phase are recorded in table 1.

Table 1.--Data recorded during spray operations

Date (June)	Treatment time A.m.	Treatment time P.m.	Acres treated	Temperature	Weather
16	5		60	54° F.	Rained lightly at 8 p.m.
17	5-7		200	53° F.	Rained hard for 2 days.
19		7-9	340	45° F.	Clear.
20	5-9		620	50° F.	Clear.
			1,220		

Most Douglas-fir tussock moth larvae were in the second instar during treatment. Several trees at higher elevations still had a few unhatched eggs and first instars on them. Some third instars had developed in the valleys.

Sampling.--Spray deposit was detected on white Kromekote cards which were held on branch tips of sample trees with clothespins. Cards were also attached to check trees to determine if spray drifted over them.

Leucophor C in the spray solution fluoresced on the cards when they were placed under a "black light." No attempt was made to correlate the number of fluorescent droplets per unit of card surface with the amount of virus deposited. Spray deposit on the cards was estimated quantitatively, such as very light, light, medium, etc.

Fifty sample trees (grand fir and Douglas-fir) were selected along sidehills and ridges within the pilot test area (fig. 8). They were 10-15 feet high, bushy, and harbored a moderate number of egg masses.

Two untreated check areas were established (fig. 8). Twenty-five infested trees (check "A") 5 to 7 feet high were transplanted about 2 miles from the test area several weeks before the test was conducted. These trees were selected from various spots within the pilot test area. All egg masses were removed. Prior to treatment, first and second instars were collected from trees near the excavation sites and liberated on the transplants (fig. 9). The number of larvae put on each transplant was based on the amount of foliage it offered as food. The reason for transplanting the above trees was to determine the degree of virus present in the test area prior to spraying. The assumption was that if polyhedrosis occurred, check larvae contacted it from natural contamination. Prespray collections indicated 9 percent of the transferred larvae were infected with polyhedrosis.

An additional 25 trees (check "B"), similar to sample trees chosen in the pilot test area, were established in an infested stand about $1\frac{1}{2}$ miles south of the test area.

One week before the pilot test area was sprayed with polyhedra, second instars were collected from each sample and check tree. Five larvae were selected from five different lower limbs on each tree. Each larva was placed in a clear, 4-ounce plastic cup (fig. 10) which contained artificial food (2). After treatment, larvae of the most prominent instar were collected, as above, at 7-day intervals. The last collection was made at the end of 42 days, at which time pupae were evident.

Collected larvae remained in the plastic cups until they died or emerged as adults. Cups were checked daily for dead larvae and to see if food was needed. Cadavers were dissected to see if they contained parasites, and a slide of their crushed body contents was examined under a compound microscope to determine the presence of polyhedra or other organisms.

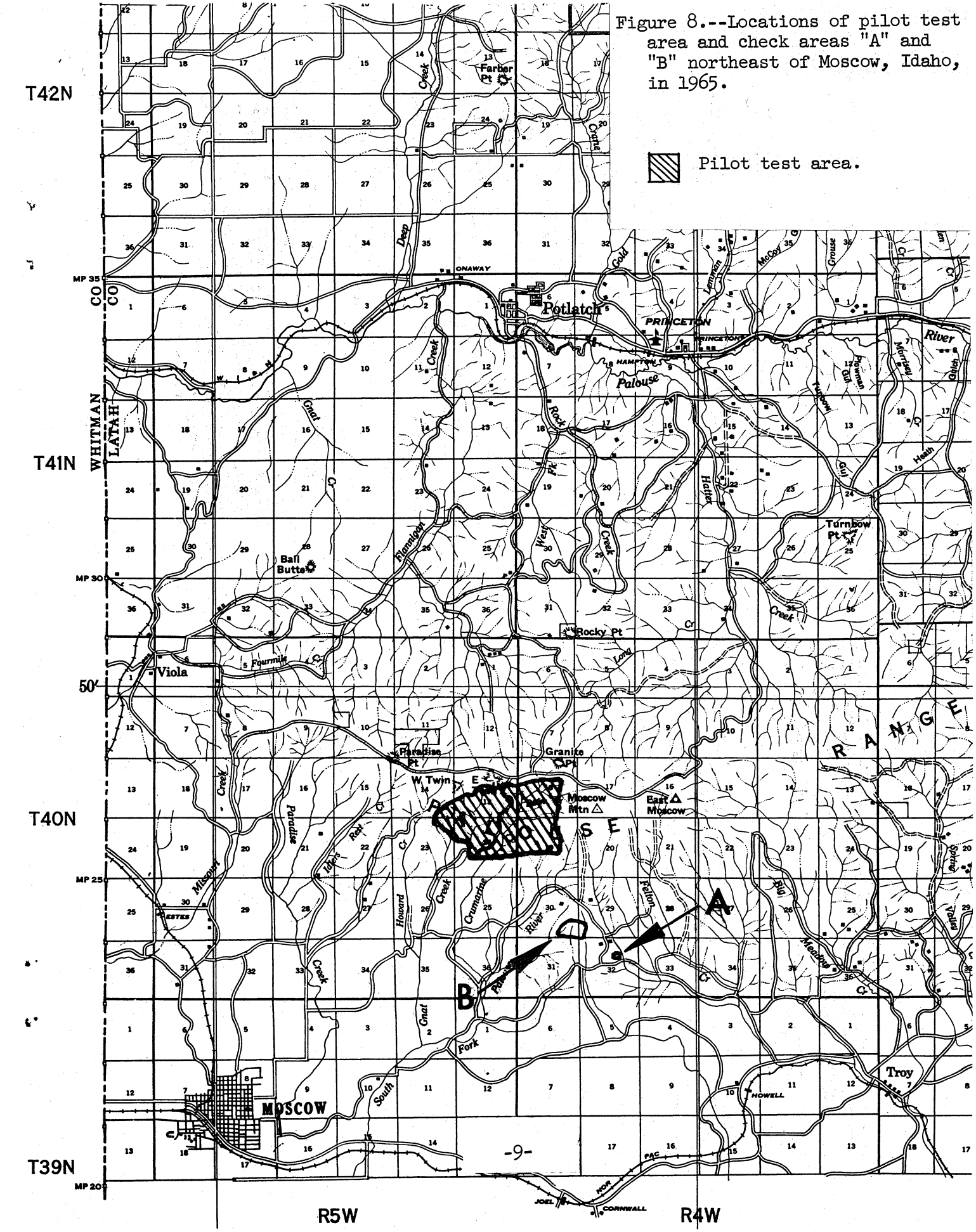


Figure 8.--Locations of pilot test area and check areas "A" and "B" northeast of Moscow, Idaho, in 1965.

Figure 9.--Liberating larvae on transplanted trees in check area "A."

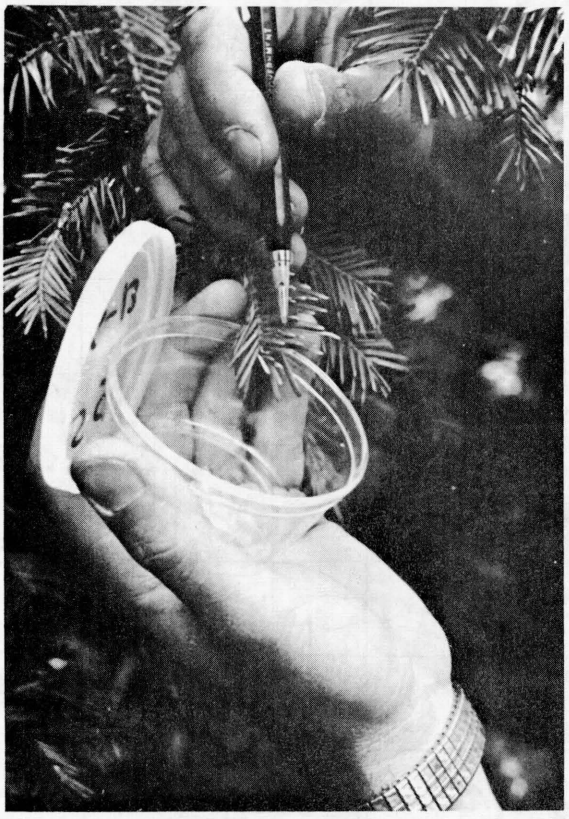


Figure 10.--Larva collected in plastic cups were reared on artificial food to determine virus incidence.

RESULTS

Data from prespray collections (tables 2, 3, and 4) indicate the percentage of larvae infected with polyhedrosis within the test area and check areas "A" and "B" was 15.0, 9.1, and 27.4, respectively. The degree of prespray infection seemed to influence results more than the application of 1 billion polyhedra per acre (fig. 11).

An analysis of variance at the 5-percent level showed mortality from polyhedrosis was significantly higher in check area "B" than in the pilot test area. Therefore, the application of 1 billion polyhedra per acre did not exert any control over the Douglas-fir tussock moth population. Data from check "A" (table 3) were not considered in the analysis because they did not seem applicable due to lower populations and poor tree vigor.

The percentage of larvae killed by polyhedrosis increased steadily in all three areas as the season progressed (fig. 11). For instance, by July 29, 80.8 percent of the population was lethally infected with the virus in check "B."

Mortality from other diseases, parasites, and unknown agents was not above 17.6 percent during any of the collection periods (fig. 12). Parasites caused most of this mortality (tables 2, 3, and 4). Percentage of parasitism was highest during the July 29 collection period (15.6 percent in the treated area). Five species of wasps killed 96.7 percent of the parasitized larvae. One species has been tentatively identified as belonging to the genus Eulimneria. The remaining 3.3 percent were parasitized by Diptera. A Tachinid fly, Carcelia yalensis Sellers, was very abundant in the field and probably killed many of these larvae.

Some adult emergence occurred in all collections from the three areas (tables 2, 3, and 4). However, many of the emerging adult males were malformed with paddlelike wings, and both sexes frequently had distorted abdomens. Neilson (5) studied the effects of a cytoplasmic polyhedrosis virus in the adults of four species of Lepidoptera. He found that the above malformations were apparent on diseased adults, and the virus infection greatly reduced their reproductive abilities. Therefore, even if a certain percentage of the Douglas-fir tussock moth population emerged in the field, many females might have been unreproductive, or the males could have had crippled wings and been unable to seek out females.

The above theory, plus additional mortality from natural factors, might explain why no current egg masses could be found in the pilot test area or check areas "A" and "B" during the fall of 1965.

Table 2.--Percentage of Douglas-fir tussock moth larvae killed by various agents in prespray and postspray collections from trees treated with polyhedrosis virus in 1965

Collection date	Instar	Number	Percent mortality: Virus	Other diseases	Parasites	Unknown	Emergence percentage
Prespray							
6/13	2d	247	15.0	2.0	8.5	5.3	69.2
Postspray							
6/26	2d	249	38.1	2.8	6.4	3.2	49.5
7/3	3d	250	32.4	1.2	7.6	2.4	56.4
7/9	4th	250	35.2	2.0	7.2	4.8	50.8
7/16	4th	250	32.8	2.8	8.4	2.8	53.2
7/22	5th	250	72.0	.4	5.2	2.0	20.4
7/29	5th	250	73.6	0	15.6	2.0	8.8

Table 3.--Percentage of Douglas-fir tussock moth larvae killed by various agents in prespray and postspray collections from untreated trees "A" during 1965

Collection date	Instar	Number	Percent mortality: Virus	Other diseases	Parasites	Unknown	Emergence percentage
Prespray							
6/13	2d	1/ 110	9.1	0.9	6.4	6.4	77.2
Postspray							
6/26	2d	110	27.4	2.7	4.5	3.6	61.8
7/3	3d	110	25.5	2.7	1.8	4.5	65.5
7/9	4th	110	31.9	--	3.6	3.6	60.9
7/16	4th	110	27.3	--	3.6	.9	68.2
7/22	5th	2/ 100	56.0	--	1.0	6.0	37.0
7/29	5th	3/ 90	67.9	2.2	5.5	2.2	22.2

1/ Three out of the original 25 transplanted trees died.
2/ Two more transplants died--which left 20.
3/ Still later, two more transplants died--which left 18.

Table 4.--Percentage of Douglas-fir tussock moth larvae killed by various agents in prespray and postspray collections from untreated trees "B" during 1965

Collection date	Instar	Number	Percent mortality				Emergence percentage
			Virus	Other diseases	Parasites	Unknown	
Prespray							
6/13	2d	124	27.4	3.2	4.0	8.1	57.3
Postspray							
6/26	2d	125	64.0	3.2	--	7.2	25.6
7/3	3d	125	54.4	--	3.2	2.4	40.0
7/9	4th	125	60.8	.8	8.8	2.4	27.2
7/16	4th	125	67.2	.8	12.8	.8	18.4
7/22	5th	125	82.4	.8	9.6	1.6	5.6
7/29	5th	125	80.8	--	15.2	1.6	2.4

DISCUSSION

Several factors may have affected results of the treatment. Deposits on spray cards indicated the spray solution was not applied uniformly over the test area. One sample tree was not sprayed, and 50 percent of the trees were sprayed lightly. The rest were sprayed moderate to heavy. The base solution of corn sirup, Leucophor C, and water was mixed on June 14. By June 19 a "scum" was detected on cheesecloth filters. More than three-fourths of the spraying was achieved on June 19 and 20. The pH of this solution was 6.8 on June 19, which will not dissolve polyhedra, but a harmful reaction might have occurred. On June 16 and 17 it rained after treatment and continued during June 18. The weather remained cool (45°-55° F.) and damp during most of June. This was not conducive for the incubation of polyhedrosis, and the rains could have washed much of the deposit off the foliage.

Thompson (9) found that 50 billion polyhedra in 1 gallon of solution per acre indicated a satisfactory control potential. This dosage rate might be considered if future Douglas-fir tussock moth outbreaks have to be controlled in Region 1.

Figure 11.--Percentage of mortality from polyhedrosis within prespray and postspray larval collections from treated area, and untreated areas "A" and "B."

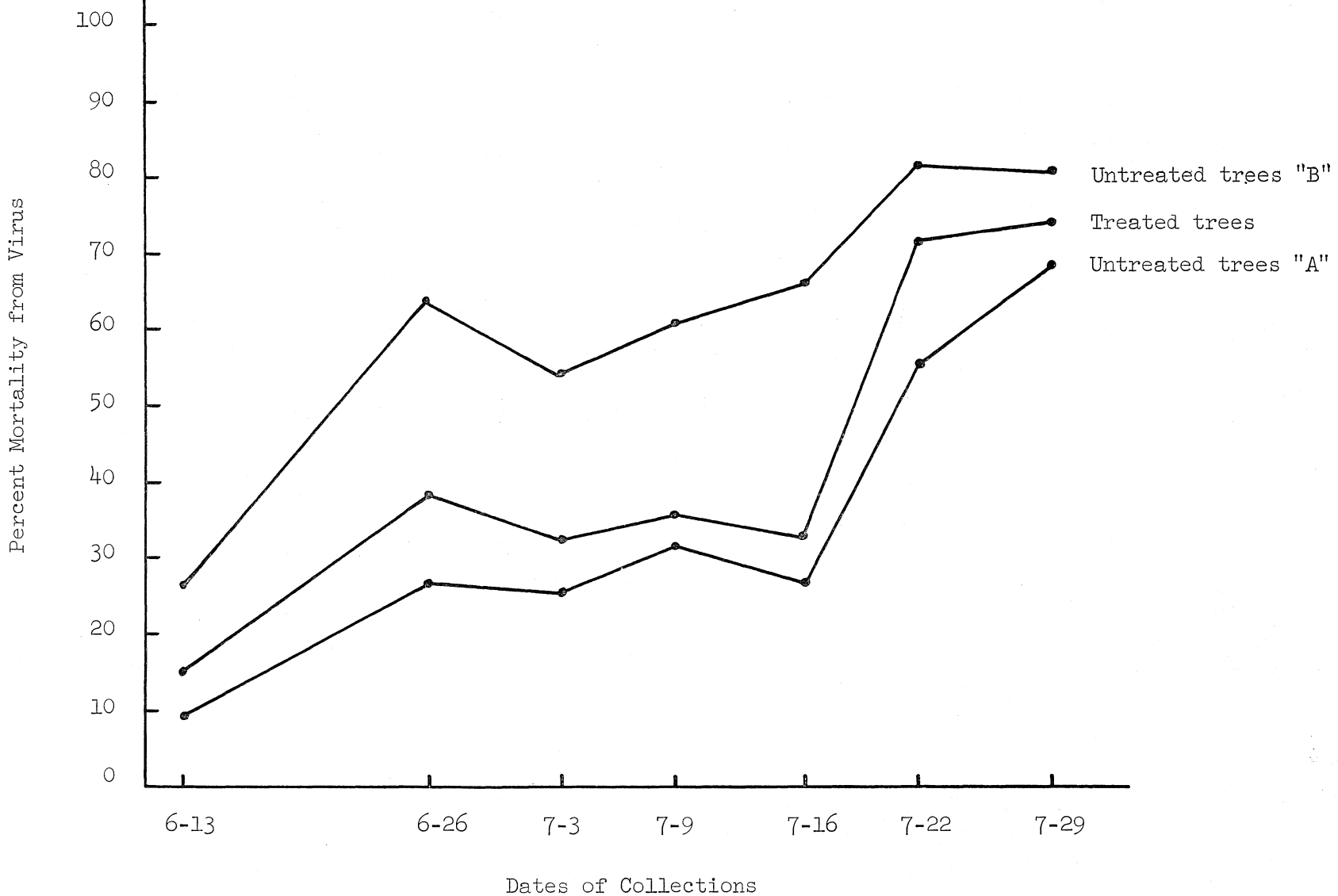
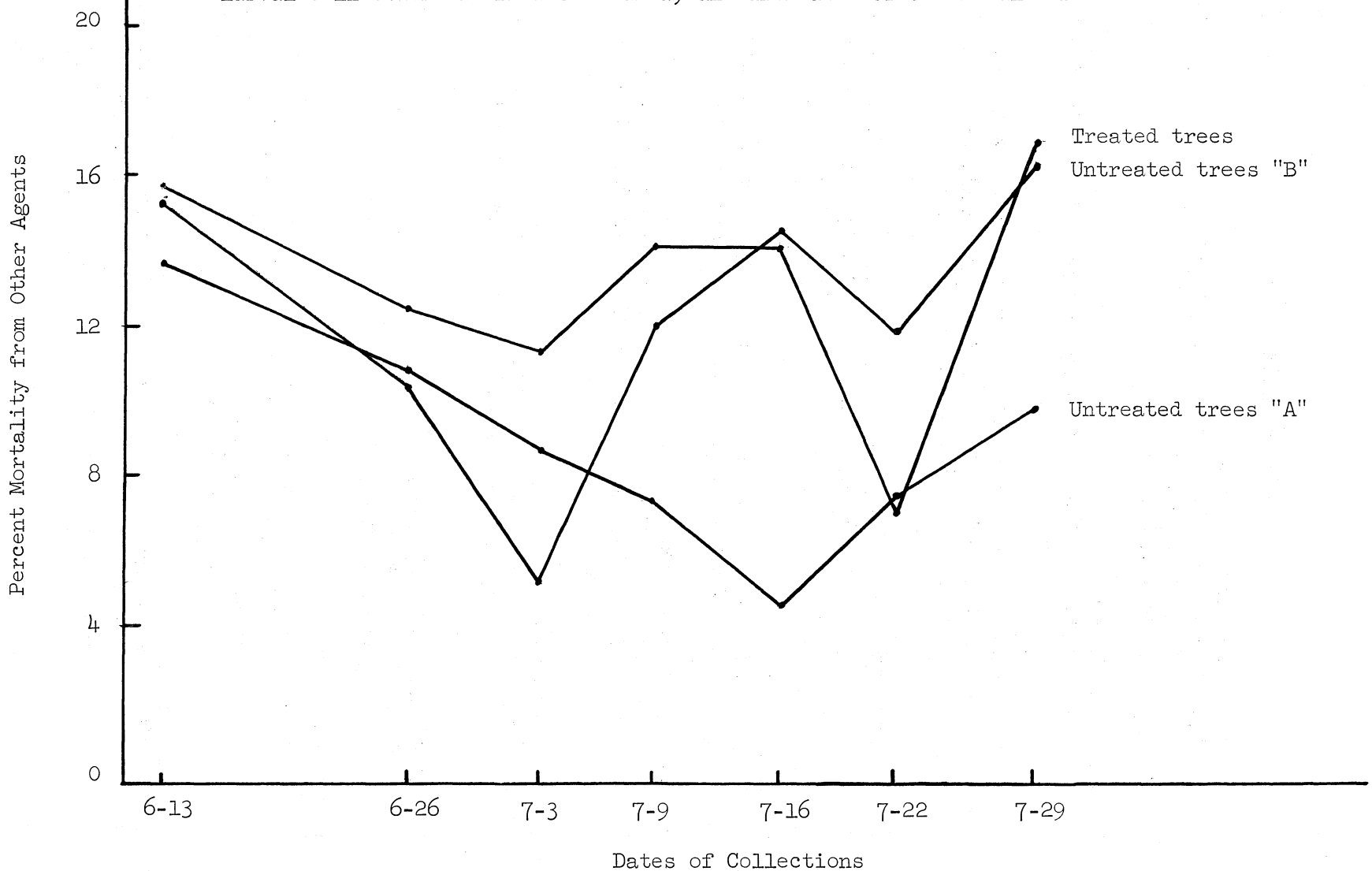


Figure 12.--Percentage of mortality from other agents within prespray and postspray larval collections from treated area, and untreated areas "A" and "B."



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